IV. DISCUSSION

The data on the effect of extreme low light intensity on chloroplast metabolism during development and senescence of primary leaves of wheat seedlings are discussed in the light of literature available mainly on sun and shade plants. Selection and characterisation in low light are made by using white light at three levels of intensity namely 625 (low), 2500 (moderate) and 20,000 lux (high). The primary leaves of wheat seedlings grown in the light intensity of 20,000 lux exhibit a sudden rise in Chl level, a dramatic decline thereafter and the level goes down remarkably during senescence phase (Fig. 2). On the other hand the level of the pigment in case of the seedlings grown in the light intensity of 2500 lux rises slowly and reaches a peak higher than that obtained with 20,000 lux light, remains in a steady state and exhibits a decline thereafter. In case of the treatment of seedlings with extreme low light intensity namely 625 lux, the pigment level remains at a relatively low level during development and steady state. Relative stability of the pigment during low than high irradiation may indicate its slow degradation in former light condition (Fig. 2).

The sudden rise, decline and a reduced level of Chl in the primary leaves of seedlings grown at high than moderate light intensity would indicate that the seedlings grown at this intensity develop characteristics, typical of sun type
plants (Lichtenthaler et al., 1981a,b). It is difficult to propose any definite mechanism to explain reduction in the level of total Chl during pigment accumulation in primary leaves of seedlings grown in high light condition. The loss of pigment due to photooxidation is one possibility. The other possibility is the decreased rate of Chl biosynthesis. When germinated seeds are exposed to high light, the sudden rise in Chl accumulation as shown in Fig. 2 could possibly be due to quick phototransformation of PChlide to Chlide. This is because of presence of a large pool of enzyme bound PChlide. Once the pool of PChlide is phototransformed to Chlide, the naked PChlide reductase becomes prone to destruction and the degradation of the enzyme starts (Santel and Apel, 1981; Kay and Griffiths, 1983). This continues until the rate of PChlide reduction matches the PChlide synthesis. So the rate limiting step in Chl biosynthesis at high light could be the PChlide synthesis, which is in turn limited by ε-amino levulenate synthesis (Nadler and Granick, 1970; Castelfranco and Beale, 1983; Bennett et al., 1987). On the other hand a slow rise during development of primary leaves and a gradual decline in the level of pigments during senescence of the seedlings grown in extreme low light intensity would indicate that the light at this intensity is a major limiting factor for pigment accumulation. The step that most likely limits the rate of Chl biosynthesis at low irradiance is the phototransformation of PChlide to Chlide, catalysed by PChlide reductase (Griffiths and Oliver, 1984).
substrate for mitochondrial respiration to generate energy. Therefore the low rate of photosynthesis is accompanied by an increased rate of mitochondrial activities in low light intensity (Kromer et al, 1993). So the enhanced rate of mitochondrial efficiency in the present work has relevance. Further experimentation is necessary in this area to confirm the precise relationship between these organelles of leaves under low light conditions.

4.2 Low Light Induced Modulation of Chloroplast Degradation During Senescence

The data on measurement of photosynthetic pigments of primary leaves of wheat seedlings grown in moderate and low light conditions as shown in Fig. 2, 3, and 4 suggest that variation in light intensity causes differential rates of degradation of photosynthetic pigments during senescence. An earlier loss of Chl in the leaves at low than moderate light would suggest that light intensity not only causes a different degree of loss of pigments but also results in variation in induction timing of leaf senescence. The variation in initiation of decay of pigments in the present work with 2 light conditions are not comparable with decay kinetics of the pigments in sun and shade type of chloroplasts as reported by Lichtenthaler et al, (1981 a, b; 1982) who have demonstrated an earlier loss of pigments in sun plants grown in high intensity of light and a delay in the induction of senescence in shade plants.
It is difficult to suggest any precise mechanism for an earlier and relatively rapid loss of pigments of the leaves in low than moderate light grown seedlings. The level of pigments at a particular stage of leaf growth is determined by its synthesis and degradation. Although degradation of pigments dominates over the synthetic process during senescence phase, synthesis of Chl, to an extent may contribute to the pigment level. In the present work the light intensity used for low light treatment could possibly cause a significant reduction in rate of Chl biosynthesis as discussed earlier and therefore fails to maintain a balance between synthesis and degradation favouring a rapid decline in synthetic ability of the pigments of the leaves in addition to its senescence induced degradation.

The other factor that may contribute to low light induced reduction of Chl could be low input of energy which may limit photosynthetic production of sugar. A reduced rate of production of sugar may not sustain the leaves for long time and therefore may contribute to the loss of the pigment during senescence (Goldthwaite and Laetsch, 1967; Thimann et al, 1977).

Relative losses of Chl a, Chl b and total Car during senescence of leaves at low and moderate light conditions are examined by calculating their ratios (Fig. 5, 6, 7, 8). Leaf senescence, irrespective of different light treatments causes a decline in Chl a/Chl b (Fig. 7) and total Chl/total Car (Fig. 5) ratios, which indicate relative loss of reaction
by absorption and fluorescence spectroscopy during the growth of the seedlings under low and moderate light intensities. A shift in absorption maxima, change in half band width and blue to red peak ratio would indicate the status of pigments on thylakoid surface in specific and structural organisation in general. Absorption spectra of chloroplasts isolated separately from the leaves of the seedlings grown for 8 days in moderate (Fig. 21A) and low light (Fig. 21B), exhibit two distinct peaks namely a red peak at 678 nm, a blue peak at 435 nm, a shoulder at 640 nm and a Car band at 470 nm. During senescence on 16th day the spectra of chloroplasts from the leaves of moderate light grown plants do not show any qualitative change. However, a reduction in the peak height, of the red absorption band as shown in Fig. 21A may be correlated with senescence induced loss in the content of Chl. On the otherhand a qualitative change like a slight increase in the height of blue absorption band as reflected in the enhancement of B/R value in case of chloroplasts isolated from senescing leaves of seedlings at low light condition (Fig. 21B) may indicate specific membrane disorganisation.

Fluorescence emission of isolated chloroplasts is measured to examine if the emission characteristics could provide information about the differential behaviour of the plants grown in two different light intensities. The fluorescence spectrum of isolated chloroplasts from leaves of
moderate light grown seedlings at steady state shows a large peak at 685 nm attributed to the emission mainly by PS II pigments, a hump at 735 nm, the emission mainly by pigments associated with PS I (Fig. 22). Fluorescence spectral characteristics of chloroplasts isolated from mature and senescing leaves of seedlings grown in moderate and weak light condition do not exhibit any significant qualitative differences (spectra not shown). However, during senescence, the intensity of fluorescence emission at 685 and 735 nm varies depending upon light conditions used (Fig. 24). The intensity of Chl a fluorescence depends on several factors. A reduced photochemical potential may lead to enhancement of Chl a fluorescence (Panda et al, 1987). A loss in pigment content and an increase in the amount of Chl uncoupled to photochemical reaction may result in decrease or increase in fluorescence intensity. A relative increase in fluorescence intensity in the chloroplasts from low than moderate light grown plants (Fig. 23, 24) may be correlated with low photochemical potential as indicated by a significant reduction in the primary photochemical reaction associated with thylakoid membrane (Table 11, 12 and Fig. 27). The other factor that could contribute to the relative increase in Chl a fluorescence in the samples of low light grown plants may be the poor development of thylakoid membrane where Chl a molecules are not properly coupled to the reaction centre. The question of variation in the content of Chl contributing
to the variation in the fluorescence intensity does not arise because fluorescence measurements are done on the basis of Chl itself.

An increase or decrease in the ratios of peak heights of $F_{685}/F_{735}$ is used as a parameter to investigate emission efficiency of PS II and PS I respectively. A relative increase in the value of $F_{685}/F_{735}$ of chloroplasts from low light grown plants in mature and senescence phases (Fig. 23, 24) would suggest relative stability of fluorescing pigments associated with PS II. Alternatively a relative increase in fluorescence emission of PS II could be correlated to a drastic decline in the rate of photochemical reactions associated with PS II fraction of thylakoid membrane as measured by DCPIP photoreduction (Fig. 26).

The differences in optical properties of chloroplasts isolated from moderate and low light grown seedlings as discussed in the light of changes in absorption and emission behaviour of the organelle are further supported by the analysis of data obtained from the measurement of excitation spectra. The excitation spectra of chloroplasts from both the samples exhibit distinct changes in different peak heights during senescence as shown in Fig. 25 and 26. The spectra show peaks of Car (485 nm), Car + Chl b (471 nm), Chl b (620 nm) and Chl a (439 and 676 nm). The changes in peak heights of spectra at these wavelengths reflect the
degree of coupling between Chl a with Chl b or Car in thylakoids. The amount of Chl a fluorescence, if the chloroplasts are excited by the wavelengths of light absorbed by accessory pigments, depends upon the proximity of Chl a with these pigments. These pigments exist in complex form on thylakoid and therefore a change in thylakoid structure will affect the spatial distribution of the pigments in the complex resulting in the alterations in the coupling of Chl a with accessory pigments. This would ultimately lead to the alteration in the peak heights of excitation spectra. Distinct variation in different peak heights of the excitation spectra of chloroplasts isolated from the leaves of low and moderate light grown plants during senescence as shown in Fig. 25, 26 suggests that light intensity may lead to variation in the organisational status of thylakoid membrane.

Compared to moderate light, low light not only causes a difference in the level of pigments and structural organisation of chloroplasts as probed by absorption and fluorescence spectroscopy, it also results in a change in primary photochemical reactions of thylakoid membrane. The primary leaves of the seedlings grown in low light exhibit a reduced level of photosynthetic efficiency as measured by DCPIP (Fig. 27) and MV (Table 12) reduction by isolated chloroplasts. During senescence of leaves of the seedlings grown in moderate light, a decline in the efficiency of whole chain electron transport involving participation of all electron transport complexes may indicate specific changes
associated with one or all individual complexes. However, the data on Asc-DCPIP supported and water supported MV reductions may propose a relative stability of PS I over PS II. On the other hand DPC supported significant restoration of loss of MV reduction with water as electron donor may indicate damage of OEC as the major limiting factor for the loss of thylakoid photochemistry during senescence.

When moderate light grown seedlings were transferred to the low light condition, a remarkable loss in electron transport efficiency of thylakoid membrane is recorded. In this case PS I and whole chain electron transport system remain unstable as noted with MV reduction by water and Asc-DCPIP couple. DPC does not restore the loss in MV reduction indicating damage of reaction centres of photosystem or complexes other than OEC. It appears, low light causes changes in different electron transport complexes that all contribute to the total loss of electron transport efficiency of thylakoids.

The electron transport efficiency of chloroplasts as measured by MV reduction with different electron donor systems remain significantly low in the primary leaves of the seedlings grown only in weak light condition starting from germination. During senescence there is a decline in Asc-DCPIP supported and water supported MV reactions. It is difficult to explain the low level of MV reduction by Asc-DCPIP couple compared to MV reduction by water and DPC in
weak light conditions. The possibility of poor access of reduced DCPIP to the site of its action for electron transport during senescence in this light condition can not be ruled out. DPC does not improve the efficiency of MV reduction when it replaces water as the donor system suggesting damage of reaction centres. On transfer of low light grown seedlings to moderate light condition there is slight improvement of electron transport efficiency as shown in Table 12.

The analysis of data in general on electron transport studies as shown in Table 11 and 12 indicates that moderate light causes a damage in OEC without any significant change in reaction centre II which is severely damaged by weak light during senescence.

The electron transport efficiency of chloroplasts from low and moderate light grown seedlings are compared with mitochondrial electron transport efficiency in these two light conditions (Table 13). It is well established that senescence is an active process and a part of plant developmental programme. Reports are available on an enhancement of respiratory rate during leaf senescence (Woolhouse and Jenkins, 1983). Since senescence needs energy at certain steps, an increase in respiratory rate is a strategy of plants to supply energy to the process. In the present work a high level of functional efficiency as probed by SDH activity of mitochondria from senescing leaves of low than
moderate light grown seedlings would suggest that low input of energy and reduced photochemical efficiency of leaves of low light grown plants are compensated by high mitochondrial efficiency as discussed earlier (Kromer et al, 1993).

4.3 Senescence of Chloroplasts Under Interacting Stress Factors

The data discussed so far indicate that some of the characteristics of the primary leaves of wheat seedlings grown at low light condition are comparable to that of low light acclimated shade plants. However, a highly reduced level of photosynthetic pigments of the leaves suggest that plants fail to adapt successfully to extreme low light condition used in the present investigation. The light at this intensity most likely is acting as a severe stress factor without any significant adaptation. Experiments are subsequently conducted to examine the interacting effects of low light with other stress factors like UV A radiation and water stress on the level of pigments during leaf senescence. Low light interaction with other stress factors has been examined because light stress cannot be considered as an isolated factor to affect photosynthetic process in plants in natural environment where several other stress factors may operate simultaneously.

The effects of interacting stress factors on pigment metabolism during leaf senescence has been examined because
senescence is a natural process and unfortunately the literature on the response of senescing leaves to these environmental factors is meagre.

The data as shown in Fig. 17, 18, 19, 20 and summarised in Table 9 indicate that both in moderate and low light grown plants, senescence induced loss in total Chl content of primary leaves is enhanced by exposure of the seedlings to UV A light and water stress conditions. Although the precise mechanism of UV A induced Chl breakdown during senescence is not known, the involvement of oxy free radicals generated by the radiation causing the loss of the pigment cannot be ruled out (Joshi et al, 1994 b). UV A irradiation is known to bring about disruption of thylakoid structure (Joshi et al, 1994 b). Disorganisation of membrane structure causes activation of chlorophyllase (Terpstra and Lambers, 1983 a, b) leading to loss of Chl. Similarly water stress has been extensively reported to result in a decline of the content of Chl of green plants which may be attributed to the loss of activities of scavenging enzymes (Baisak et al, 1994). The stress induced stimulation of pigment loss during senescence corroborates with the findings of Jefferies (1994), who has demonstrated reduced level of Chl content in potato leaves in water stress conditions. The stress induced stimulation of loss of Chl and Car whether by UV A radiation or by water stress during leaf senescence, is greater in low than moderate light intensity (Table 9). This would mean that the plants under low light
are more prone to other environmental stress factors than the plants in moderate light conditions. These findings also support the proposition that low light used in the present work is acting as a stress and effect of its interaction with other factors is additive.